Rapid Total Bacterial RNA Extraction Kit

Code No.： NG322S 50 T

NG322M 100 T

Contents	storage	50 T	100 T
lysozyme	4 ℃	25 mg	50 mg
Buffer RL	4 ℃	50 ml	100 ml
Buffer RE	RT	25 ml	50 ml
Buffer RW	RT	10 ml	25 ml
		Add absolute alcohol before use	
Spin Columns RA	RT	50	100
RNase-free H_2O	RT	10 ml	10 ml
Collection tubes（2 ml）	RT	50	100

Important Note：

All solutions should be clear. If the ambient temperature is low, the solutions may form precipitates and should not be used directly. In such cases, they be heated in a 37 ℃ water bath for a few minutes to restore clarity. Improper storage at low temperatures (4 ℃ or -20 ℃) can precipitation in the solutions and affect the performance, therefore both transportation and storage should be carried out at room temperature (15 ℃ – 25 ℃).

Introduction：

The improved guanidinium isothiocyanate/phenol one-step method (NGzol method) lyses cells and inactivates RNases, then total RNA selectively adsorbed onto a silica matrix membrane in a centrifuge column under high chaotropic salt conditions. Subsequently, through a series of rapid washing and centrifugation steps, deproteinizing solution and washing buffer impurities such as cell metabolites and proteins. Finally, low-salt RNase-free water elutes the pure RNA from the silica matrix membrane.

Safety Information:

Lysis buffer RL and RE contain irritating and harmful compounds. Wear latex gloves during operation to avoid contact with skin, eyes and clothing. If contact with skin or eyes occurs, rinse with plenty of water or saline.

Note: self-prepared

To extract bacterial RNA, first prepare TE buffer containing lysozyme (10mM Tris-HCl, 1mM EDTA pH 8.0). The final concentration of lysozyme in TE is: G- bacteria: add 1mg/ml. Incubate at 37 ℃ for about 5 minutes; G+ bacteria: add 5mg/ml. Incubate at 37 ℃ for about 10-15 minutes.

Operation Procedure:

Tip: Before the first use, please add absolute alcohol in the buffer RW and mark it.

1. Centrifuge and collect 1-2 ml of bacterial culture (approximately 10^8 - 10^9 cells) into a 1.5 ml centrifuge tube, remove the supernatant as much as possible, and ensure that the residual supernatant does not exceed 20 μl.
2. Depending on the type and number of cells, completely resuspend the cells in 100 μl (<$5x10^8$ cells) of lysozyme must be added to TE first), or resuspend them directly in TE, and then use a clean pipette tip to add a small amount of lysozyme into it.

3. Incubate at room temperature (15-25 ℃) for 5 min to break the cell wall. Vortex for 10 seconds every 2 min.
   Note: The difficulty of cell wall lysis varies for different bacteria. Generally, for Gram-negative bacteria, the above conditions are sufficient and this step may even be omitted. However, for certain Gram-positive bacteria, which are harder to lyse, the lysozyme concentration needs to be increased to 35 mg/ml and the incubation time to 10 minutes. If Staphylococcus aureus is used, nlysostaphin needs to be added to 1 mg/ml, and incubated at 37 ℃ for 5-15 min. In summary, different types of bacteria vary in the ease of cell wall lysis. For species that are difficult to lyse, the type of enzyme, working, and incubation temperature and time need to be adjusted according to the user's specific conditions. In addition, methods such as combined Proteinase K digestion can also be used to facilitate wall lysis.
4. Add 1 ml RL for per 50–100 mg sample. Usually, the volume of tissue sample should not exceed 10% of the volume of RL. Incubate at 15-30 ℃ for 5 min.
   Optional step: Centrifuge the sample at 12,000 rpm for 10 min at 4 ℃. Transfer the supernatant to a fresh micro-centrifuge tube.
   Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. RNA remains in the upper aqueous phase after centrifugation.
5. Add 200 µl of chloroform. Vortex for 15 sec. Incubate for 2-3 min at room temperature. Centrifuge at 12,000 rpm for 10-15 min at 4 ℃. The mixture separates into three phases, a lower yellow phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Pipette the aqueous phase (about 600 µl) out into a new tube.
6. Add 1/2 volume of ethanol, invert to mix (a precipitate may form at time). Transfer the resulting solution and any possible precipitate into a micro-adsorption column RA (the adsorption column is placed inside a collection tube).
7. Centrifuge for 30 s at 12,000 rpm. Discard the flow-through and set the Spin Column RD back into the Collection Tube.
8. Add 500 µl of Buffer RE to the Spin Column and centrifuge for 30 sec, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
9. Add 500 µl of Buffer RW to the Spin Column and centrifuge for 30 sec, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
10. Repeat step 9.
11. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
12. Add 30-80 µl of RNase-free H_2O to the center of the membrane, let the column stand for 2 min, then centrifuge for 1 min at 12,000 rpm. Stored at -70 ℃.